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Review Article

Immunological and Clinical Effects of Vaccines Targeting p53-Overexpressing Malignancies

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Approximately 50% of human malignancies carry p53 mutations, which makes it a potential antigenic target for cancer immunotherapy. Adoptive transfer with p53-specific cytotoxic T-lymphocytes (CTL) and CD4⁺ T-helper cells eradicates p53-overexpressing tumors in mice. Furthermore, p53 antibodies and p53-specific CTLs can be detected in cancer patients, indicating that p53 is immunogenic. Based on these results, clinical trials were initiated. In this paper, we review immunological and clinical responses observed in cancer patients vaccinated with p53 targeting vaccines. In most trials, p53-specific vaccine-induced immunological responses were observed. Unfortunately, no clinical responses with significant reduction of tumor-burden have occurred. We will elaborate on possible explanations for this lack of clinical effectiveness. In the second part of this paper, we summarize several immunopotentiating combination strategies suitable for clinical use. In our opinion, future p53-vaccine studies should focus on addition of these immunopotentiating regimens to achieve clinically effective therapeutic vaccination strategies for cancer patients.

1. Introduction

Despite recent progress in surgical, chemotherapeutic, and radiotherapeutic approaches, cancer is still difficult to treat and cure, especially in patients with advanced stage of disease. Therefore, new therapeutic strategies are required. One of the new treatment strategies is immunotherapy targeting tumor-associated antigens (TAA).

Mutation of the p53 tumor-suppressor gene is a frequent event in human oncogenesis. The role of the p53 gene has been reviewed extensively by Vogelstein and Vousden [1–3]. P53 mutations found in tumors were shown to abrogate the regulatory function of p53 on the cell cycle. Moreover, many mutations lead to an increased half-life of the otherwise rapidly degraded p53 protein and thereby to accumulation of this protein in cells [4]. Other tumor suppressor genes

often lose their expression after mutation, but the point mutated p53 protein is often more stable and therefore overexpressed in tumor cells [5, 6]. p53 degradation can also be promoted directly through binding to viral proteins or deletions promoting presentation for T cell recognition [1, 2].

CD8⁺ cytotoxic T-lymphocytes (CTLs) are the most important effector cells for antitumor immune responses. They recognize TAA-derived peptides that are processed and presented on the tumor cell surface in association with major histocompatibility complex (MHC) class I molecules, leading to killing of tumor cells [7]. Processing of the intracellular p53 protein by the proteasome will result in presentation of p53-derived peptides in the context of MHC class I molecules at the tumor cell surface. CD4⁺ T-helper (Th) cells play an important role in orchestrating and

sustaining the local immune attack by CTL [8, 9]. In contrast, CD4⁺FoxP3⁺ regulatory T cells (Tregs) impede antitumor immunity by inhibiting CTL activation [10, 11].

The search for widely expressed tumor antigens as targets for MHC class I restricted CTLs is of great importance for the development of T cell-mediated immunotherapy of cancer. As persistent overexpression of p53 or induced T cell presentation is present in ~50% of a wide variety of cancers, a large group of patients would benefit from p53 directed immunotherapy.

Since p53 is a self-antigen expressed at low levels in normal cells, immunogenic tolerance might hinder the use of wild type p53 as a tumor antigen for immunotherapeutic approaches. Moreover, the idea of targeting a nonmutated wild-type p53 gene with a vaccine may be counterintuitive. So far induction of p53-specific CTL and Th cells with the capacity to eradicate p53-presenting tumors without inducing clinical nor immunopathological damage to normal tissue has been observed in different mouse models, despite the fact that wild-type p53 is expressed in normal tissue [12–14]. This tumor selectivity could be explained by the increased p53 protein expression resulting from p53 mutation [13]. Alternatively, insufficient antigen display in normal tissues by the MHC class I molecule in combination with lack of or proper costimulation and downregulatory chemokine and cytokine conditions might protect against the destruction by the potentially autoreactive wild-type p53-specific CTL [15, 16]. Consequently, wild-type p53-specific CTLs are able to discriminate between p53-presenting tumor cells and normal tissue, indicating that widely expressed autologous molecules such as p53 can serve as a target for CTL-mediated immunotherapy of tumors [17].

In humans, spontaneous MHC class I restricted p53-specific CTL [18, 19], MHC class II restricted p53-specific proliferating Th cells [20, 21], and p53 antibody responses have been observed [22, 23]. Furthermore, several naturally processed human wild-type p53-derived epitopes in both MHC class I and MHC class II have been identified [17]. The presence of cellular and humoral immune responses against p53 shows that tolerance is not complete for this self-antigen. In particular CD4 T cell tolerance, based on mouse observations, is far from profound [24].

On the basis of these preclinical results, which indicate the occurrence of p53-directed immune responses in cancer patients, several clinical trials have been performed with vaccines targeting p53. These studies have, however, generally not yet evolved past phase I/II studies.

In this paper the immunogenicity and clinical efficacy of p53-specific active immunotherapy in human cancer is evaluated to assess the potential of this treatment modality for cancer. Furthermore, we propose a few straightforward clinically applicable combination strategies to improve clinical efficacy of p53-directed immunotherapies.

2. Clinical Trials of p53 Peptide Cancer Vaccine

Several phase I/II immunization trials using p53 immunogens have been conducted so far (Table 1). We have summarized the observed immune and clinical responses

in cancer patients, induced by the p53-vaccine (Table 2). Next, we provide a more detailed account of the studies, categorized by the different vaccination strategies.

2.1. Viral Vector-Based Vaccines. Viral vectors encoding recombinant transgenes for TAAs (such as p53) capable of infecting host cells can elicit a tumor-specific immune response against the transgene product. Recombinant viral vector-vaccines encoding full-length TAA may contain epitopes for both CD4⁺ T-helper (Th) cells and CD8⁺ cytotoxic T-lymphocytes (CTLs). The clinical advantage of this vaccination strategy therefore is that the MHC type of the individual patient does not need to be considered (reviewed in [37–40]). Several clinical studies on viral vector-based vaccines encoding p53 have been conducted.

In a pilot study, Kuball et al. immunized six advanced stage cancer patients with a recombinant replication-defective adenoviral vector encoding human full-length wild-type p53 [25]. Neither tumor responses nor anti-p53 responses were observed; however, all patients showed an adenoviral immune response. This strong anti-adenoviral-specific response may have competed out the p53-specific response. Clinical tumor responses were assessed by imaging diagnostics using National Cancer Institute response criteria. Three months after initial immunization, 4 patients had stable disease. After followup of 7–16 months only one patient had stable disease.

Based on preclinical results in mice and rhesus macaques, Menon et al. performed a phase I/II clinical study involving vaccination of end-stage colorectal cancer patients with a recombinant canarypox virus (ALVAC) encoding wild-type p53 [26, 27]. Patients were immunized intravenously with an increasing dosage of ALVAC-p53. From this study, it appeared that this modality is safe and capable of stimulating p53-specific Th1 (IFN- γ) responses in several of these patients. One out of 16 patients showed stable disease for a short period of time after immunization with the highest dose. Fever was the only vaccine-related adverse event. The authors conclude from this trial that repeated immunizations are probably necessary to obtain good clinical responses. Again, antivector responses were observed in all patients after vaccination which, by antigenic competition, may have prevented robust anti-p53 immune responses.

In a phase I/II study, Antonia et al. tested a cancer vaccine consisting of dendritic cells transduced with the full-length wild-type p53 gene delivered via an adenoviral vector [28]. Significant p53-specific T cell responses to vaccination was found in 13 out of 25 patients (52%) in IFN- γ ELISPOT assays. In 7 out of 12 HLA-A2 positive patients, an increase in frequency of CD8⁺ T cells that secrete IFN- γ in response to targets pulsed with an HLA-A2 restricted p53 peptide were found. Four out of 10 patients with a detectable preimmunization level of anti-p53 antibody developed a positive p53-specific T cell response to vaccination. No link was found between the presence of CD4⁺FoxP3⁺ regulatory T cells (Tregs) and p53-specific T cell responses to vaccination in the patient's blood before or after vaccination, despite the assumption that Tregs downregulate the antitumor immune response. Objective clinical responses were observed in

TABLE 1: P53-targeting vaccines in human cancer.

Author	Year	Study	Vaccine	Tumor site	n	Disease status	Previous treatment	Imm*	Ref
Kuball et al.	2002	Pilot study	recombinant virus	urogenital-, lung cancer, malignant schwannoma	6	advanced disease	unknown	4	[25]
Menon et al.	2003	Phase I/II	recombinant virus	colorectal cancer	16	metastatic disease	chemotherapy/radiation therapy/other	3	[26, 27]
Antonia et al.	2006	Phase I/II	recombinant virus	small cell lung cancer	29	extensive/recurrent disease	chemotherapy (1 to ≥ 3 regimens)	± 3	[28]
Svane et al.	2004	Phase I	peptide pulsed DC	breast cancer	6	progressive/metastatic disease	chemotherapy/radiotherapy/endocrine therapy	10	[29]
Svane et al.	2007	Phase II	peptide pulsed DC	breast cancer	26	progressive/metastatic disease	chemotherapy (1–5 regimens)/endocrine treatment (1–3 regimens)	10	[30]
Lomas et al.	2004	Phase I	short peptide	breast, colorectal, non-small-cell lung, renal, prostate, head- and neck, hemangiopericytoma, esophageal cancer	14	NED/metastatic /recurrent disease	yes	4	[31]
Rahma et al.	2010	Phase II	short peptide/peptide pulsed DC	ovarian cancer	21	NED	surgery/chemotherapy	≤ 31	[32–34]
Leffers et al.	2009	Phase II	long peptides	ovarian cancer	20	recurrent disease	surgery/chemotherapy	4	[35]
Speetjens et al.	2009	Phase I/II	long peptides	colorectal cancer	10	metastatic disease	surgery/chemotherapy	2	[36]

NED: no evidence of disease. * Number of immunizations.

TABLE 2: Immune and clinical response p53-targeting vaccines.

Author	Year	Humoral response ¹	ELISPOT	Cellular response ² <i>Proliferation</i>	Immunohistochemistry ³	Clinical response ⁴	Toxicity	Ref
Kuball et al.	2002	no anti-p53 specific Abs	no p53-specific response	not analyzed	3/6 positive	4/6 SD 2/6 PD	CTC I, local reaction, fever	[25]
Menon et al.	2003	pre 7/15 post 10/15	4/15 PR	1/15 PR	not analyzed	1/16 SD	CTC I/II, fever	[26, 27]
Antonia et al.	2006	pre 10/22 post 10/22	16/28 PR	p53-specific proliferation not analyzed	not analyzed	1/29 PR* 7/29 SD* 21/29 PD*	CTC I/II	[28]
Svane et al.	2004	not analyzed	4/6 PR	not analyzed	3/6 positive	2/6 SD* 2/6 PD* 2/6 MR/UR*	mild/moderate local reaction/flu-like symptoms	[29]
Svane et al.	2007	not analyzed	8/22 PR	not analyzed	11/26 positive	8/19 SD* 11/19 PD*	CTC I/II, local reaction, flu-like symptoms	[30]
Lomas et al.	2004	pre 0/6 post 1/6	0/6 PR	2/6 VIR	14/14 positive	Not analyzed	CTC I/II, local reaction, nausea, arthralgia	[31]
Rahma et al.	2010	not analyzed	10/19 PR	not analyzed	21/21 positive	3 NED 1 SD 16 PD	CTC III/IV	[32–34]
Leffers et al.	2009	pre 8/20 post 9/20	18/18 PR	14/17 PR	9/20 positive	2 SD* 18 PD*	CTC I/II, local reaction	[35]
Speetjens et al.	2009	not analyzed	6/9 PR	7/10 VIR	6/10 positive	3/10 NED 7/10 PD	CTC I/II, local reaction, flu-like symptoms	[36]

¹ Pre- and postimmunization levels of anti-p53-specific antibodies. ² p53-specific T-lymphocytes induced by immunizations, PR: positive response, VIR: vaccine-induced response. ³ p53-staining of primary tumor samples. ⁴ SD: stable disease, PD: progressive disease, MR: mixed response, UR: unconfirmed regression, PR: partial response, NED: no evidence of disease, *all according to Response Evaluation Criteria in Solid Tumors.

61.9% of 21 patients treated with second-line chemotherapy directly after immunization. This result provides direct clinical evidence that cancer vaccines may be most effective not as a single modality, but rather in a close combination with other methods of treatment, specifically, chemotherapy. This observed effect could be explained by a number of potential mechanisms, such as down-regulation of the effect of tumor-produced immunosuppressive factors that prevent CTLs from killing tumor cells by chemotherapy [41], or up-regulation of p53 in tumor cells, which can make them more susceptible to recognition by CTLs [42], or lastly, chemotherapy may make tumor cells more susceptible to the cytotoxic effect of CTLs through a perforin-independent increase in permeability to granzyme B released by the CTLs [43].

Collectively, viral vector-based vaccines encoding p53 are well tolerated in early-phase clinical trials with minimal toxicity. Limited p53-specific immune responses might be due to antigen competition, as all patients had strong antivector responses. Future studies on viral vector-based vaccines should focus on the use of prime-boost strategies with different vectors delivering p53. This strategy overcomes the antigenic competition in priming with viral vectors. Viral vector recombinant Semliki Forest virus, which is not strongly affected by vector-neutralising antibodies therefore, has exquisite potency in homologous prime-boost immunization regimens [44].

2.2. Dendritic Cell-Based Vaccines. It is important to investigate the character of the p53-specific T cell responses, because p53-based vaccination of patients should be aimed at boosting only the desired Th1-type immunity, while stimulation of Th2-type or Tregs should be avoided [45]. This finding would argue in favour of application of a p53-specific vaccination using a delivery mode specifically stimulating the anti p53 (CTL) and Th1 responses. Autologous dendritic cells (DC) expressing the antigen of interest could be one of these ways (reviewed in [46–48]). Dendritic cells are highly potent professional antigen-presenting cells (APCs). Therefore, antitumor vaccines have been designed, using DCs generated on clinical scale loaded with synthetic MHC binding peptides known to stimulate peptide-specific CTLs, like p53.

Svane et al. reported on their phase I immunization study in breast cancer patients with p53 peptide pulsed DC [29]. Autologous dendritic cells were pulsed with three wild-type and three modified HLA-A2 restricted p53 peptides combined with an MHC class II binding peptide (PADRE). Patients received ten subcutaneous immunizations with at least 5×10^6 peptide-pulsed dendritic cells combined with 6 mIU/m² interleukin 2 (IL-2). Two out of six patients had a clinical response and three out of six developed p53-specific T cell responses (including the two patients with a clinical response), without significant toxicity.

The phase II study performed by Svane et al. [30] was carried out in direct continuation of their phase I study using the same vaccination regime as described above. Only five out of 26 patients completed all ten planned immunizations due to rapid progression of disease or death. Positive

immunohistochemical staining of p53 by the primary tumor was found more frequently in patients achieving stable disease during treatment, indicating an effect of p53-specific immune therapy. However, immunohistochemical staining for p53 might underestimate the patients' ability to present p53 at its tumor cell surface, as tumors in which p53 is inactivated indirectly through binding to viral proteins for example, will not score positive for p53, but can be recognized by CTLs [1, 2]. In most cases, an increase in the number of p53-specific CTLs during vaccination was measured; however, a tendency towards a more marked decline at late time points after vaccination was observed. However, these heavily pretreated metastatic breast cancer patients with a high tumor burden are not the ideal patient group to translate p53-specific activation of the immune system into significant tumor regression.

Dendritic cell-based vaccines are laborious in production and restricted to individual patients, but have the advantage that DCs are highly efficient APCs [49]. A significant fraction of the advanced stage breast cancer patients obtained disease stabilization and induction of p53-specific immunity during p53-DC vaccination. Type and maturation status of DCs are issues to be solved in future studies with this vaccination approach. Moreover, further clinical studies should be performed at an earlier stage of disease with progression-free survival or overall survival as an endpoint.

2.3. Peptide-Based Vaccines

2.3.1. Short Peptides. Since the first identification of a defined tumor-specific CTL epitope, the concept of immunizing cancer patients with a single synthetic peptide epitope has been elaborated (reviewed in [50–52]). The relatively poor immunogenicity of peptide epitopes requires them to be injected together with adjuvants. Important advantages of short peptide vaccination are its defined nature and easy manner to synthesize.

Lomas et al. performed a phase I trial targeting several p53-overexpressing solid cancer types in 14 patients with an idiotypic vaccine, composed of a pool of eight peptides derived from the complementarity determining regions (CDRs) of human anti-p53 antibodies admixed with granulocyte-macrophage colony-stimulating factor (GM-CSF) [31]. None of the trial patients was found to have vaccine-specific, IFN- γ -secreting T cells as assessed by ELISPOT assay. However, a vaccine-induced response was observed in 2 out of 6 patients in the proliferation assay. Clinical responses were not registered and only CTC I/II toxicities were observed.

Rahma et al. compared subcutaneous wild-type p53 epitope (264–272) vaccination with intravenous peptide-pulsed DC administration in 21 ovarian cancer patients combined with IL-2 adjuvant in a randomised phase II study. IL-2 administration resulted in directly induced expansion of Tregs and in grade II/IV adverse events in both arms of the study, which was thereafter omitted from the regimen for these patients [32–34]. P53-specific T cells were observed in approximately 70% of patients, irrespective of whether short peptides or peptide-pulsed DCs were used.

Recent insights in short peptide vaccination have indicated that vaccination with short exact MHC class I binding peptides dissolved in chemical adjuvants, in contrast to peptide-pulsed DCs, is suboptimal mainly because short peptides load exogenously onto MHC class I molecules, including those of nonprofessional antigen-presenting cells [53].

2.3.2. Long Peptides. Another vaccination strategy is the use of long peptides encoding the whole p53 protein. The advantage of using long peptides is that if delivered in the appropriate adjuvant (with APC stimulatory capacity), all potential MHC class I and MHC class II epitopes within the delivered peptides will be processed and presented to host T cells. These long peptide vaccines are independent of MHC-binding motif prediction or processing algorithms and can be administered to subjects independent of their MHC type (reviewed in [53]).

A phase I/II trial using wild-type p53-derived synthetic long peptides (SLP) in ovarian cancer was performed by Leffers et al. [35]. Twenty patients with recurrent elevation of CA-125 were included and immunized with 10 overlapping p53-SLP in Montanide ISA51. IFN- γ producing p53-specific T cell responses were induced in all patients who completed the vaccination-scheme as measured by IFN- γ ELISPOT. Vaccine-induced p53-specific T cells are mediated predominantly by Th2-cells as determined by cytokine bead array and are capable of migration into immunization sites. The number of Tregs remained constant before and after immunization. Stable disease was observed in 2 out of 20 patients, although no relationship was determined with vaccine-induced immunity.

Speetjens et al. used the same p53-SLP vaccine (Leffers et al.) in a phase I/II trial, vaccinating ten metastatic colorectal cancer patients [36]. P53-specific T cells isolated from the vaccination site were characterised as Th cells which displayed a mixed T-helper 1 and 2 cytokine profile with varying percentages of IFN- and IL-2 producing p53-specific T cells as determined by cytokine bead array. No overt induction of p53-specific Tregs after p53-vaccination was found. Furthermore, in 6 out of 9 patients, strong proliferative p53-specific T cell responses were observed in blood samples taken ~6 months after the last vaccination.

Peptide based-vaccines have the advantage that antigen-specific immune responses can be easily monitored as a tool to improve the vaccine or vaccination strategy [52]. However, vaccination with short peptides is far from optimal because it can lead to immunological tolerance of the immunizing antigens because T and B cells, in contrast to properly activated DC, lack the costimulatory surface molecules required for appropriate effector CTL generation [54–58]. In addition, immunizations with short-peptide vaccines may induce outgrowth of antigen loss variants of the tumor [59]. Furthermore, a single peptide epitope induces either Th cells or CTL and responses to such epitopes are limited to patients with specific MHC types capable of presenting the peptide used [53]. Limited humoral, cellular and clinical responses were shown in patients immunized by short-peptide vaccines.

In contrast, IFN- γ producing p53-specific T cell responses were induced in the majority of patients receiving long-peptide vaccination. This is probably attributable to the fact that the T cell epitopes in the long peptide vaccine are efficiently processed and presented by dendritic cells and that the response induced by this vaccine is not restricted to one MHC type. Despite the induction of p53-specific T cell immunity in vaccinated patients, the p53 long peptide vaccines so far have not induced clinical efficacy. Long peptide vaccines targeting p53, therefore, should be combined with other forms of treatment to eliminate potential mechanisms of immune failure.

3. Perspectives

Thus far, p53-targeting therapeutic vaccination strategies in cancer patients including administration of recombinant viral vectors, peptide pulsed dendritic cells, short peptide and long peptide vaccines have not shown consistent and/or convincing clinical efficacy.

Whereas some of these vaccines, in particular viral vectors and short peptides, have intrinsic shortcomings, a likely explanation for the lack of efficacy is that, despite induction of p53-specific CD4⁺ T-helper (Th) cells and the recruitment of CD8⁺ cytotoxic T-lymphocytes (CTLs) to the tumor, a robust antitumor response is not accomplished due to immunoregulatory mechanisms counteracting effective T cell-mediated tumor cell killing. T cells that effectively home to tumor metastases can be dysfunctional, pointing toward immunosuppressive mechanisms in the tumor microenvironment [60]. T cell anergy due to insufficient B7 costimulation, extrinsic suppression by regulatory myeloid and regulatory T cell populations, inhibition by ligands such as programmed death ligand-1, metabolic dysregulation by enzymes such as indoleamine-2,3-dioxygenase, and the action of inhibitory factors such as TGF- β have all been implicated in the lack of efficacy [45, 61].

Because of the disappointing clinical results induced by the p53-vaccines, we can conclude that the immunogenicity of these vaccines needs to be enhanced by improving the robustness of the induced effector T cell responses and by effectively disrupting the counterproductive immunoregulation [62]. It may also be useful to simultaneously target additional tumor antigens [63]. Below we discuss several straightforward clinically applicable methods that have been proposed to augment immunogenicity and clinical efficacy of immunotherapeutic vaccines.

3.1. Eliminating Regulatory T Cells by Cyclophosphamide. As mentioned above, the observed lack of clinical efficacy may be partly attributed to the presence of CD4⁺FoxP3⁺ regulatory T cells (Tregs). It is becoming apparent that immunotherapy itself can induce and/or boost Tregs and that these vaccine-induced Tregs are associated with treatment failure [64–68]. Immunosuppression mediated by Tregs is a major hurdle for successful tumor immunotherapy as Tregs suppress antigen-specific T cell responses [60, 65–67]. Strategies to eliminate or suppress Tregs to improve clinical efficacy of immunotherapy vary from treatment

with commonly used chemotherapeutic agents, such as cyclophosphamide, fludarabine, or COX-2 inhibitors, next to direct targeting of Tregs by monoclonal antibodies [69–76].

Low-dose cyclophosphamide is easy to incorporate into a clinical setting. Dosages of cyclophosphamide used in combination with immunotherapy are generally insufficient for cytotoxic reductions of tumor burden, but reduce numbers of Tregs and impair their functionality without deleting other immune cells [69, 77–79]. Furthermore, a cohort study in metastatic pancreatic cancer showed an enhanced induction of antigen-specific T cells in patients pretreated with cyclophosphamide compared to patients who were not pretreated with cyclophosphamide. Additionally, median overall survival of patients treated with cyclophosphamide was almost twice as high as that of patients who did not receive cyclophosphamide. This was similar to results obtained with second-line therapy for metastatic pancreatic cancer [80]. Although the number of circulating Tregs in the patient group vaccinated with the p53-SLP by Leffers et al. is relatively low (7.0%), their presence and recruitment to the tumor may nevertheless foster tolerance to the tumor. We have started a new clinical trial in which p53-SLP immunization is combined with low-dose cyclophosphamide to test whether this increases immunity and clinical activity.

3.2. Immunopotentiality by Anti-CTLA-4. Another immunopotentiality strategy that has been used in the clinical setting is blockade of cytotoxic T-lymphocyte antigen-4 (CTLA-4) aiming to counteract inhibitory signals in order to induce antitumor immunity. CTLA-4 is a costimulatory molecule expressed on activated T cells that delivers an inhibitory signal which reverses the T cell response, resulting in anergy [81]. Two human anti-CTLA-4 monoclonal antibodies (mAbs), MDX-010 (ipilimumab) and CP-675,206 (tremelimumab), have thus far been used in clinical trials with encouraging results in patients with melanoma, lymphoma, and urothelial carcinoma of the bladder [74, 82–85]. Anti-CTLA-4 mAbs are well poised to be combined with other therapies. Moreover, these antibodies may enhance the effectiveness of other therapies like cancer vaccines when used in combination. Therefore, several clinical trials on antitumor regimens added anti-CTLA-4 to their treatment regime, aiming to improve clinical efficacy in the participating patients [86–88].

Anti-CTLA-4 mAbs have shown antitumor activity; however, accumulating evidence indicates that anti-CTLA-4 mAbs paradoxically increases the number of Tregs, thereby hampering the effect of anti-CTLA-4 [89]. This has stimulated interest in designing clinical trials using anti-CTLA-4 mAbs in combination with Treg controlling strategies to improve clinical outcome. Several promising preclinical studies combining anti-CTLA-4 with Treg depletion have been conducted so far [90–92].

The studies of combined immunopotentiality low-dose cyclophosphamide and anti-CTLA-4 provide the foundation for integrating immunotherapy with other targeted therapies for the treatment of patients with advanced stage cancer.

3.3. Immunostimulation by Chemotherapeutic Regimens. The interaction of tumor cell death due to chemotherapy on one hand and induction of antitumor immune responses induced by this cell death on the other hand might be essential to achieve the optimal result in tumor eradication [93–95]. It is postulated by Zitvogel et al. that activation of the calreticulin exposure pathway is an important mechanism of activation of the immune system after treatment with classical therapies like chemotherapy [96]. They thought that chemotherapy in general results in a strong reduction of major components of the immune system and thereby harming the immune system ready to attack the tumor does not hold true anymore. Evolving evidence shows the opposite. Immunotherapy in combination with chemotherapy might be a very effective strategy as induction of long-lived antigen-specific memory T cells recently has been identified [97]. Cisplatin next to paclitaxel and doxorubicin, drugs often used in gynaecologic malignancies, reportedly make tumor cells more susceptible to Granzyme B-dependent killing by cytotoxic T cells [43]. It is attractive to use these immunomodulatory effects of chemotherapy by combining it with p53-specific immunization.

3.4. New Vaccination Strategy: Multi-Epitope Vaccines. Most clinical studies included in this paper targeted only p53, limiting the use of such vaccines to those patients with (over)expression of this specific tumor antigen. Furthermore, tumor cells might lose antigens and therefore display a reduced susceptibility to vaccine-induced immunity in the course of the vaccinations. Immunization using a cocktail of antigens has been proposed as a “universal” vaccine strategy [98]. As solid tumors often show heterogeneous protein expression, multi-antigen vaccines may have greater therapeutic potential which can compensate for tumor antigen-loss variants [63, 99]. The ability to target multiple antigens may also improve the immunogenicity of therapeutic vaccines. We believe that addition of other tumor antigens to the p53-vaccine might ultimately result in an enhanced clinical effect [98], particularly because the CTL repertoire against p53 based on mouse studies and observations in patients, appears to be more deeply tolerized than the Th cell repertoire [24]. Addition of immunotherapy against antigens that more readily elicit tumoricidal CTL responses may therefore fully exploit the excellent ability of p53 vaccination to elicit Th cell responses.

Thus far, several clinical trials targeting multi-antigens have been conducted. Kirkwood et al. reported that the effect of a multi-epitope melanoma vaccine tested in a phase II trial is correlated with prolonged survival in metastatic melanoma patients. Addition of immunomodulatory cytokines had no beneficial effect on prognosis [100]. A multi-antigen vaccine tested in prostate cancer patients resulted in a long-term stable disease [101].

Recently, a p53 comprising multi-epitope vaccine has been administered to malignant melanoma patients in a phase I/II clinical trial. Results of the DC-vaccine pulsed with p53, survivin and telomerase-derived peptides in combination with low-dose IL-2, have been published by Trepkiakas et al. [102]. This group previously targeted p53

in a DC vaccination trial as described in this paper [29, 30]. Due to this new multi-antigen pulsed DC-vaccine, stable disease correlated with prolonged survival suggesting a clinical benefit. Nevertheless, significant changes in Treg frequencies during treatment were seen and ascribed to IL-2 administration. Consequently, IL-2 was removed from their DC vaccination strategy and replaced by low-dose cyclophosphamide in an ongoing clinical trial in melanoma patients in order to enhance the immune and clinical responses.

Addition of multiple antigens in an immunotherapeutic vaccine will enhance the barrier against escape of antigen loss variants of the tumor and will exploit more fully the antitumor CTL potential of the patient. Future studies on multi-epitope immunotherapy, moreover applicable in a higher percentage of patients, are expected to result in a significantly enhanced efficacy of anticancer immunotherapy.

4. Conclusion

Over the past decade, several studies on p53-vaccines for immunotherapeutic treatment of cancer patients have been conducted. Different vaccination strategies varying from viral vectors, dendritic cells, and short and long peptides have been used. Of these vaccination modalities, viral vectors and short peptides suffer from major drawbacks. Although peptide-loaded DC and long peptides have induced reasonably strong p53-specific immune responses, in particular CD4⁺ T cell responses, robust clinical responses so far have failed to materialize. In this paper, we point out that the limited clinical efficacy dictates further exploration of new immunization strategies. P53-vaccines can easily be combined with low-dose cyclophosphamide, anti-CTLA-4, chemotherapeutic regimens, or other tumor antigens, as immunopotential treatment modalities. An integrative immunotherapeutic strategy combining “up-front” Treg cell ablation followed by p53 vaccination may limit generation of new tumor-sensitized Tregs and therefore, might improve the clinical responses in cancer patients. Moreover, addition of multiple antigens to the p53-vaccine will make it applicable in a higher percentage of patients and will exploit the anticancer T cell response. Future studies will be needed to establish the best combination of therapy and to identify cancer patients most likely to respond to combined anti-p53 therapies.

Conflict of Interests

The authors declare that there is no conflict of interests.

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